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[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Steroidal Sapogenins. XLVI. Side Chain Structure of 20-Isosapogenins[2,3]

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Dehydration of 20-hydroxytigogenin acetate (I) gave the unsaturated olefin II. The structure of II was established by hydroxylation with osmium tetroxide followed by cleavage with periodic acid to give IV. Catalytic hydrogenation of II in neutral solvents gave 20-isotigogenin (VII).

The stereochemistry of the sapogenin spiroketal side chain has been a subject of considerable interest in recent years. As the result of contributions from a number of laboratories, the structure of the spiroketal side chain of naturally occurring sapogenins seems well established and it is now generally agreed that such compounds differ only at C_{24}.[4a,b]

A new class of sapogenins obtained by treatment of a pseudosapogenin with acetic acid or dilute hydrochloric acid was obtained recently almost simultaneously in several laboratories.[5a-e] The stereochemistry of the spiroketal side chain of this class of compounds, which we wish to call 20-isosapogenins, has not been settled. This paper reports a partial synthesis of 20-isotigogenin acetate and of 20α-hydroxy-20-isotigogenin acetate which establishes in unequivocal fashion the side-chain structure of 20-isosapogenins of the 25D-series.

Oxidation of 20-isotigogenin acetate[6] with chromium trioxide in acetic acid gave a mixture of 3β,16β-dihydroxy-allopregnane-20-one 3-acetate 16-γ-methylglutarate and a new hydroxylated sapogenin (I). Compound I was obtained in approximately 45% yield and was separated easily by crystallization or chromatography from the acidic side chain cleavage product. Formulation of I as a probable 20-hydroxysapogenin was based on the following evidence. The optical rotation and infrared spectrum of I indicated that the spiroketal system was intact. The analytical constants for carbon and hydrogen were in agreement for a sapogenin with one additional hydroxyl group, substantiated by the infrared spectrum which showed a strong band at 3510 cm.$^{-1}$. This hydroxyl was tertiary as indicated by the fact that it could not be further oxidized, nor acetylated with hot pyridine–acetic anhydride and was dehydrated easily under mild conditions. On this basis compound I was at this stage designated as 20-hydroxytigogenin acetate with unspecified stereochemistry at C_{20}.

Dehydration of I with thionyl chloride in pyridine gave a new unsaturated sapogenin, formulated as $\Delta^{20(21)}$-tigogenin acetate (II). The latter was the key intermediate in all our subsequent work. The carbon and hydrogen analysis of II was in accord with the loss of one mole of water in going from I to II. This was confirmed by the infrared spectrum which showed absence of hydroxyl bands and new bands at 3080, 1797, 1665 and 903 cm.$^{-1}$ indicative of unsaturation, probably of a $R_1R_2{=}CH_2$ type.[7] That the unsaturated grouping was indeed a $C^{20(21)}$-methylene was proved as follows. Reaction of II with osmium tetroxide in benzene gave the diol-monoacetate III which, on

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(2) Paper XLV, Wall and Serota, THIS JOURNAL, **79**, 6481 (1957).

(3) Presented at second Delaware Valley Regional ACS Meeting, Philadelphia, Pa., February 5, 1958, and at 133rd National ACS Meeting, San Francisco, Calif., April 13–18, 1958.

(4) (a) Pertinent literature through 1955 is cited in a paper by M. E. Wall, *Experientia*, **11**, 340 (1955); (b) R. K. Callow and P. N. Massy-Beresford, *Chemistry & Industry*, 1146 (1956).

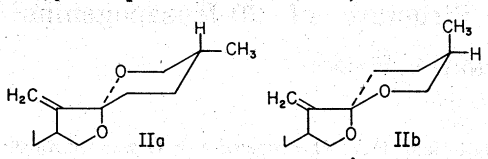
(5) (a) M. E. Wall, C. R. Eddy and S. Serota, THIS JOURNAL, **76**, 2849 (1954); **77**, 1230 (1955); (b) J. B. Ziegler, W. E. Rosen and A. C. Shabica, *ibid.*, **76**, 3865 (1954); **77**, 1223 (1955); (c) R. K. Callow and V. H. T. James, *Chemistry & Industry*, 691 (1954); (d) D. H. W. Dickson, J. Elks, R. M. Evans, A. G. Long, J. F. Oughton and J. E. Page, *ibid.*, 692 (1954); (e) R. K. Callow, D. H. W. Dickson, J. Elks, R. M. Evans, V. H. T. James, A. G. Long, L. F. Oughton and J. E. Page, *J. Chem. Soc.*, 1966 (1955).

(6) M. E. Wall and H. A. Walens, THIS JOURNAL, **77**, 5661 (1955).

(7) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen & Co., Ltd., London, 1954, pp. 31–47.

treatment with periodic acid, was smoothly cleaved to give 20-nor-20-ketotigogenin acetate (IV), and formaldehyde. Compound IV was also obtained, but in poor yield, by ozonolysis of II. The carbon and hydrogen analysis of IV was in agreement with the assigned structure. Moreover, a new ketone band at 1762 cm.$^{-1}$ appeared in the infrared spectrum of IV, indicative of a ketone in a five-membered ring.[8] The reaction sequence thus described clearly established the fact that a methylene group is found in compound II and that this group must be attached to C_{20}. It follows that the new hydroxyl group in I is attached to C_{20}.

We are now in a position to examine the side chain stereochemistry of II. Since this compound is derived from tigogenin, a 25D-sapogenin,[9] by methods which preclude inversion at C_{25},[10] there are only two possibilities for the spiroketal side chain of II as indicated. The infrared spiroketal "fingerprint" bands[11a,b] of II in the region 1000–850 cm.$^{-1}$ (*cf.* Experimental section) were identical



IIa IIb

qualitatively and quantitatively with those of tigogenin acetate with the exception that the spectrum of the former has one additional band at 903 cm.$^{-1}$, due to the methylene group. The fact that II has the identical system of infrared bands found in all natural 25D-sapogenins[11a,b] seems to us a compelling argument for structure IIa. Consistent with this structure is the fact that II was not affected by prolonged equilibration in ethanolic hydrochloric acid, whereas a compound with structure IIb might be expected on equilibration to give the theoretically more favorable conformation shown in IIa. Further convincing arguments for IIa will be developed at a later stage in this paper.

Molecular models of II show that the rear or α-side is relatively unhindered at C_{20} and C_{21}, whereas approach of entering groups from the β side would be severely restricted. It seems most probable that the classical "rule of rear side attack" is applicable to the attack of the bulky osmium tetroxide on the $C^{20(21)}$-double bond.[12] Consequently the diol-monoacetate must have the C_{20}-stereochemistry shown in formulation III. Acetylation of III with acetic anhydride in pyridine gave the 20-hydroxy-3,21-diacetate (V). Treatment of III with *p*-toluenesulfonyl chloride in pyridine gave the oily 21-tosylate VI, characterized by infrared spectrum but otherwise not isolated in crystalline form. Lithium aluminum hydride reduction of VI, followed by mild acetylization gave an excellent yield of I. Since the route III → VI → I involved

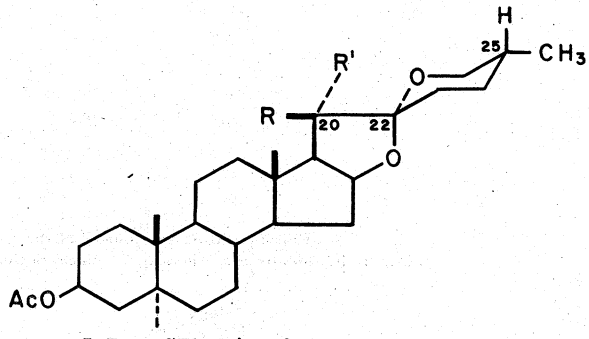
(8) L. J. Bellamy, ref. 7, p. 128.

(9) The route proceeds *via* tigogenin → pseudotigogenin → 20-isotigogenin → 20-hydroxytigogenin → $\Delta^{20(22)}$-tigogenin.

(10) Only prolonged heating with ethanolic hydrochloric acid affects this asymmetric center, *cf.* M. E. Wall, S. Serota and L. P. Witnauer, THIS JOURNAL. **77**, 3086 (1955).

(11) (a) C. R. Eddy, M. E. Wall and M. K. Scott, *Anal. Chem.*, **25**, 266 (1953); (b) R. N. Jones, E. Katzenellenbogen and K. Dobriner, THIS JOURNAL, **75**, 158 (1953).

(12) T. F. Gallagher and T. H. Kritchevsky, *ibid.*, **72**, 882 (1950).



I, R = CH_3, R′ = OH

II, R, R′ > = $=CH_2$

III, R = CH_2OH, R′ = OH

IV, R, R′ > = $=O$

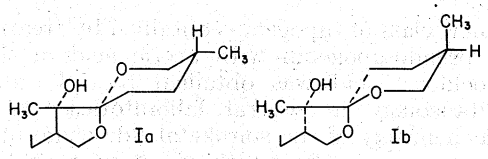
V, R = $CH_2—O—\overset{O}{\overset{\|}{C}}—CH_3$, R′ = OH

VI, R = $CH_2—OSO_2C_6H_4CH_3$, R′ = OH

VII, R = CH_3, R′ = H

Fig. 1.

reactions which could not affect the stereochemistry at C_{20}, the stereochemistry of I is established with the attached hydroxyl group being alpha and the methyl group beta. Establishment of the C_{20}-configuration of I permits a reassessment of the stereochemistry of the side chain in this series. Since I and II have been inter-converted by methods which preclude isomerization of C_{20}, C_{22} and C_{25}, the two compounds must have identical spiroketal stereochemistry. Hence compound I must have structure Ia or Ib as shown.



Ia Ib

A study of the infrared spectrum of I in the hydroxyl region permits an unequivocal solution to the problem. The hydroxyl band in I appears at 3510 cm.$^{-1}$ and is of an intensity approximately twice that of an ordinary unbonded hydroxyl. The location[13] and intensity[14] of this hydroxyl band indicated that the hydroxyl group in I was strongly hydrogen bonded. This bonding was intramolecular as shown by the fact that neither the location nor intensity of the hydroxyl group was changed in a series of dilutions between 0.2 and 10.0 g./liter.[14]

Referring to formulations Ia and Ib above, molecular models indicate that a compound with structure Ia should exhibit strong hydrogen bonding to the ring F oxygen atom and that no hydrogen bonding should occur in a compound with structure Ib. The data thus found in conjunction with the previous evidence cited for the structure of $\Delta^{20(21)}$-tigogenin acetate (II) seems to establish conclusively the fact that the spiroketal side chain in

(13) R. N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954).

(14) L. J. Bellamy, ref. 7, pp. 83–94.

the series under discussion is identical at C_{22} and C_{25} with natural 25D-sapogenins.

Finally, hydrogenation of II with platinum oxide (Adams catalyst) under neutral conditions gave exclusively 20-isotigogenin acetate (VII), identical with the product previously obtained by cyclization of pseudotigogenin in acetic acid.[6] This finding indicated a completely rear (alpha) side attack on the $C^{20(21)}$-double bond.[15] Since it has been previously shown that catalytic hydrogenation and osmylation take the same steric course,[12] the structural assignments of the 20-hydroxyl group in compounds I, III, V and VI seem firmly established. Moreover, since the conversion of II to VII was carried out under neutral conditions and since we had previously shown that 20-isosapogenins are not affected by hydrogenation under such conditions,[6] it follows that VII must have the same C_{22} and C_{25} configuration and conformation as the other members of this series, *i.e.*, that of the natural 25D-sapogenins. Hydrogenation of II under acidic conditions gave, as might be expected, dihydropseudotigogenin acetate identical to the product obtained by similar treatment of 20-isotigogenin acetate.[6]

The evidence presented in this paper seems convincing that 20-iso, 25D-sapogenins have the identical C_{22}- and C_{25}-configurations and conformations as natural sapogenins of the 25D series. The data are in agreement with our previous work based on interpretation of optical rotation data[4a] and pseudomerization studies.[2] If our views are correct, then the formation of this class of sapogenins from pseudosapogenins must involve a non-concerted *cis* cyclization *via* a relatively stable carbonium ion.[16]

Experimental[17]

3β,20α-Dihydroxy-5α,20β,22β,25D-spirostane 3-Acetate (I).[18]—(a). A solution of 55 g. of 20-isotigogenin acetate[6] and 13 g. of sodium acetate in 1500 ml. of glacial acetic acid was cooled to 12°. A solution of 22 g. of chromium trioxide in 50 ml. of 80% acetic acid was added dropwise with stirring to the steroid solution over a period of 0.5 hour at such a rate that the temperature did not exceed 15°. The solution was then allowed to come to room temperature and remained at this temperature for 0.5 hour. Two liters of water was added and the mixture extracted three times with one-liter portions of ether. The ethereal extracts were combined, washed successively with water, sodium bicarbonate solution, and water, then dried with anhydrous sodium sulfate. The ether was concentrated to approximately one-third the original volume at which stage a crystalline product (I) precipitated, and was filtered. On crystallization from methanol 25 g. of I, m.p. 214–225°, was obtained, yield 44%. Several recrystallizations from methanol gave the analytical sample, hexagonal plates, m.p. 234–236°, $[\alpha]^{25}D$ −71.0°; infrared spectrum shows strong hydroxyl band at 3510, 1735 (acetate) and bands at 991(s), 926(s), 905(w), 865(w) cm.$^{-1}$ (spiroketal). *Anal.* Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found: C, 73.92; H, 10.24.

(b) An improved method for making compound I is exemplified as follows: 18.0 g. of 20-isotigogenin acetate was oxidized with chromium trioxide as described above. The oxidation mixture was diluted with two volumes of water, filtered, and the precipitate washed well with water and dried *in vacuo* at 50°. Chromatography on 50 g. of Florisil in benzene solution gave 2.0 g. of impure I in benzene eluates. Elution with chloroform gave 9.7 g. of pure I. Elution with alcohol–benzene gave an amorphous acid which on alkaline cleavage gave 3β-hydroxy-16-allopregnen-20-one.

3β-Hydroxy-5α,22β,25D-spirost-20(21)-ene 3-Acetate (II).—To a solution of 5.0 g. of I in 200 ml. of pyridine cooled in an ice-bath was added 25.0 ml. of thionyl chloride dropwise with stirring. The solution was allowed to come to room temperature. After standing two hours it was poured over cracked ice. Extraction with ether, followed by the usual work-up and crystallization from methanol, gave 2.6 g. of crystalline II, m.p. 185–192°, yield 54%. The analytical sample was obtained by several further crystallizations from methanol, long rods, m.p. 190–191°, $[\alpha]^{25}D$ −89.5°; λ 211 mμ (ethanol), ε 1220, infrared spectrum showed no hydroxyl bands, 1735(s) cm.$^{-1}$ acetate; 3080(w), 1797(w), 1665(w) and 903(s) cm.$^{-1}$ bands due to an $R_1R_2=CH_2$ structure and the following spiroketal fingerprint bands 984(s), 938(w), 925(m), 897(s) and 865(w) cm.$^{-1}$ identical to those of authentic tigogenin acetate. *Anal.* Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 76.42; H, 9.98.

3β,20α,21β-Trihydroxy-5α,20β,22β,25D-spirostane 3-Acetate (III).—A solution consisting of 1.0 g. of II and 0.6 g. of osmium tetroxide in 30 ml. of benzene and 0.7 ml. of pyridine was stored in the dark at room temperature for 12 days. The mixture was then treated in succession with 50 ml. of water, 23 ml. of benzene, 33 ml. of methanol, 5 g. of sodium bisulfite and 5 g. of potassium bicarbonate with stirring for four hours. The benzene layer was drawn off, the aqueous residue extracted several times with ether, and then all the organic solvent fractions were combined, dried over anhydrous sodium sulfate and concentrated. Preliminary chromatography on a short column of Florisil gave on elution with benzene a small fraction, m.p. 189–193°, unreacted II. Elution with chloroform gave the major fraction 0.6 g., m.p. 210–215°. Rechromatography on Florisil gave, on elution with benzene containing 10% chloroform, 0.42 g. of crystalline III, m.p. 210–216°, which on recrystallization from heptane gave the analytical sample, double m.p. 208–210°, followed by appearance of new crystals, m.p. 217–220°, $[\alpha]^{25}D$ −66.9°, infrared spectrum, 3615 and 3520 cm.$^{-1}$, 21- and 20-hydroxyl groups, respectively; 1736 cm.$^{-1}$, 3-acetate; 988(s), 928(m), 920(m), 914(m), spiroketal bands. *Anal.* Calcd. for $C_{29}H_{46}O_6$: C, 70.98; H, 9.45. Found: C, 71.25; H, 9.30.

3β,20α,21β-Trihydroxy-5α,20β,22β,25D-spirostane 3,21-Diacetate (V).—A solution of 0.08 g. of III in 2 ml. of pyridine was mixed with 2 ml. of acetic anhydride and allowed to stand overnight at room temperature. Water was added and the product extracted with ether. After the usual work-up, 0.04 g. of V was obtained as plates from methanol, m.p. 230–233°, $[\alpha]^{25}D$ −43.5°; infrared spectrum shows a single 3480 cm.$^{-1}$ band, 20-hydroxyl; 1736(s) cm.$^{-1}$, 3,21-diacetate; and the following spiroketal bands 990(s), 925(s), 905(w). There was insufficient sample for carbon and hydrogen analysis.

3β-Hydroxy-20-oxo-5α,20-nor,22β,25D-spirostane 3-Acetate (IV).—(a). A solution of 0.14 g. of III in 20 ml. of ethanol was mixed with a solution of 0.07 g. of periodic acid in 0.7 ml. of water. The mixture was allowed to stand in the dark for four days. Water was added followed by ether extraction in the usual manner. Chromatography on Florisil gave on benzene elution 0.1 g. of crystalline product which on methanol crystallization gave 0.07 g. of leaf-like crystals, m.p. 189–191°, $[\alpha]^{25}D$ −87°; infrared spectrum shows following bands, hydroxyl absent, 1762(s), 1735(s) cm.$^{-1}$, 20-ketone and 3-acetate, respectively; 1000(s), 922(s), 906(m), 869(m) spiroketal bands. *Anal.* Calcd.

(15) Referring again to the two formal structural possibilities for compound II, *i.e.*, IIa or IIb, it is apparent that catalytic hydrogenation of the former from the front (beta) side would have necessarily given the known tigogenin acetate; similar hydrogenation of the latter would have resulted in a new sapogenin; neither could give 20-isotigogenin acetate.

(16) G. Stork and A. W. Burgstahler, THIS JOURNAL, **77**, 5068 (1955), have shown, and cite other pertinent references which indicate, that in the cyclization of certain polyenes non-concerted cyclizations may occur if condensations are favorable for the production of stable carbonium ions. The tertiary C_{22} carbon of pseudosapogenins is adjacent to an oxygen atom giving ideal conditions for the formation of a stable carbonium ion during acid-catalyzed cyclizations.

(17) Infrared spectra were obtained in carbon bisulfide solution, 10 g./liter; optical rotations were conducted in chloroform using a 2-dm. tube, 12.5 g./liter. We wish to thank C. S. Fenske and C. R. Eddy for infrared data, S. Serota for the optical rotations and R. Y. Fitz for carbon and hydrogen analyses.

(18) For basis of formal nomenclature, particularly at C_{22}, *cf.* "Tentative Rules for Steroid Nomenclature," *Compt. rend. Dix-Huitieme Conf.*, Zurich, 20–28 Juillet, 1955, pp. 190–198; *cf.* also footnote 15b in reference 2.

for $C_{28}H_{42}O_5$: C, 73.32; H, 9.23. Found: C, 73.04; H, 9.15.

Formaldehyde Test.—Prior to adding water to the periodate reaction mixture, a 2-ml. aliquot was removed and added to a solution containing 0.06 ml. of 2 *N* sulfuric acid in 8 ml. of water. The solution was distilled slowly until 3 ml. of distillate were collected. The distillate was mixed with 5 ml. of chromotropic acid reagent and heated on a steam-bath. A purple color developed indicating formaldehyde was present. A similar test run on a reagent blank treated identically to the periodate oxidation of the steroid gave a negative test.

(b) A solution of 0.5 g. of II in 30 ml. of ethyl acetate was cooled to −60° in a Dry Ice–acetone-bath. Ozone was passed through the solution until a persistent blue color was noted. The solvent was then removed *in vacuo*. The residue was taken up in benzene and chromatographed on Florisil. The benzene eluates contained 0.08 g. of IV, identical to the product obtained by periodate oxidation of III.

Conversion of III to I.—A suspension of 0.15 g. of III in 0.3 ml. of pyridine was warmed. To this was added 0.15 g. of *p*-toluenesulfonyl chloride and the mixture heated on a steam-bath until all the solids dissolved. The mixture was allowed to stand overnight at room temperature, and decomposed by addition of four drops of water followed by heating on the steam-bath. The mixture was taken up in ether, washed successively with dilute hydrochloric acid, sodium carbonate, and water and then dried over anhydrous sodium sulfate. A clear, white oil was obtained which was characterized by infrared spectrum as being the crude 21-tosylate of compound III. This oil, designated as VI, was dissolved in 25 ml. of dry ether and added dropwise to a refluxing suspension of 0.4 g. of lithium aluminum hydride in 25 ml. of ether. Refluxing was continued for four hours and the lithium aluminum hydride decomposed by addition of water followed by 10% sodium hydroxide solution. The aqueous suspension was extracted with ether in the usual manner. The product was acetylated with pyridine–acetic anhydride at room temperature. Following the usual work-up, methanol crystallization gave 0.07 g. of product, m.p. 234–235°, identical to I.

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